JOURNAL OF VIROLOGY, Sept. 2006, p. 8582–8592 Vol. 80, No. 17
0022-538X/06/$08.00+0 doi:10.1128/JVI.00031-06

Cross Talk between PML and p53 during Poliovirus Infection: Implications for Antiviral Defense

Mathieu Pampin,[1] Yannick Simonin,[2] Bruno Blondel,[2] Yann Percherancier,[1] and Mounira K. Chelbi-Alix[1]*

CNRS FRE 2944, Institut Lwoff, 7 rue Guy Moquet, 94801 Villejuif, France,[1] and Institut Pasteur, 75724 Paris, France[2]

Received 4 January 2006/Accepted 12 June 2006

PML nuclear bodies (NBs) are dynamic intranuclear structures harboring numerous transiently or permanently localized proteins. PML, the NBs' organizer, is directly induced by interferon, and its expression is critical for antiviral host defense. We describe herein the molecular events following poliovirus infection that lead to PML-dependent p53 activation and protection against virus infection. Poliovirus infection induces PML phosphorylation through the extracellular signal-regulated kinase pathway, increases PML SUMOylation, and induces its transfer from the nucleoplasm to the nuclear matrix. These events result in the recruitment of p53 to PML NBs, p53 phosphorylation on Ser15, and activation of p53 target genes leading to the induction of apoptosis. Moreover, the knock-down of p53 by small interfering RNA results in higher poliovirus replication, suggesting that p53 participates in antiviral defense. This effect, which requires the presence of PML, is transient since poliovirus targets p53 by inducing its degradation in a proteasome- and MDM2-dependent manner. Our results provide evidence of how poliovirus counteracts p53 antiviral activity by regulating PML and NBs, thus leading to p53 degradation.

PML nuclear bodies (NBs) are dynamic intranuclear structures harboring numerous transiently or permanently localized proteins (28). PML is directly induced by interferons (IFNs) (8, 40). Its expression is essential for IFN-induced cell death (44) and is critical for antiviral host defense (33). Other proteins induced by IFN, such as Sp100, PA28, and p53 or proteins that regulate p53 activity are also found in these structures (13, 33). PML is the NBs' organizer (20, 26), since in acute promyelocytic leukemia and in PML knockout −/− cells, PML NBs do not exist, and proteins normally recruited to these structures are no longer NB-associated and present a diffuse nuclear localization outside the NBs.

The PML protein belongs to the RBCC (RING finger, B boxes, and coiled-coil) domain (also termed TRIpartite motif [TRIM]) which is expressed in at least seven different isoforms (PML I to VII, according to nomenclature by Jensen et al. [21]). All isoforms contain the N-terminal region comprising the RBCC motif but differ in their C-terminal region. PML is expressed in the diffuse nuclear fraction of the nucleoplasm and in NBs. In PML-transfected (47) or IFN-treated (32) cells, the majority of PML is found in the nucleoplasm. PML NB formation requires, in addition to the presence of an RBCC motif, a specific posttranslational PML modification, the covalent linkage of the small ubiquitin-related modifier (SUMO) to lysines 65, 160, and 490. PML SUMOylation (reviewed in reference 38) was initially thought to be responsible for targeting PML toward NBs (26), as suggested by the observation that trioxide arsenic (As2O3) treatment increased PML SUMOylation and the size of NBs by recruiting PML from the nucleoplasm to the NBs (26, 47). Nuclear matrix-targeting of PML was shown, however, to occur independently of SUMOylation (23), even though this modification of PML is important for formation of NBs and the recruitment of specific proteins to these structures (20, 25). The functions of the different PML isoforms remain unclear. We have shown that expression of the PML III isoform confers resistance to vesicular stomatitis virus (VSV), influenza virus, and human foamy virus (HFV) (9, 33, 34). PML deficiency also renders mice more susceptible to lymphocytic choriomeningitis virus and VSV infections, further attesting to the antiviral activity of PML in vivo (4). The well-known disorganization of PML NBs by various viruses (33, 34) may represent part of the general viral strategy to counteract IFN action.

Poliovirus, the etiological agent of paralytic poliomyelitis, belongs to the *Picornaviridae* family (46). This virion is composed of a single-stranded RNA molecule of positive polarity surrounded by an icosahedral capsid composed of four proteins, VP1 to VP4. Poliovirus causes paralysis due to the destruction of motor neurons (3), a consequence of poliovirus replication (10). During paralytic poliomyelitis, it has been shown that poliovirus multiplication and central nervous system injury are associated with apoptosis in the mouse model (14). In vitro, poliovirus infection can induce apoptosis in different cell lines (5, 36). Recent reports indicate, first, that the viral protease-polymerase precursor 3CD can enter the nucleus of poliovirus-infected cells (39) and, second, that poliovirus 3C induces cleavage of the p65-Rel1A subunit of NF-κB (29) and p53 degradation (45).

p53 is important for the control of cell growth arrest, senescence, and cell death (15). The p53 protein is tightly regulated. Under normal conditions it is kept labile, but upon exposure to stress, p53 transiently accumulates in an active form in the nucleus. This nuclear traffic, necessary for p53 activation, is mediated under certain circumstances by PML. p53 is recruited to the PML NBs in response to Ras activation, UV

* Corresponding author. Mailing address: CNRS FRE 2944, Institut Lwoff, 7 rue Guy Moquet, Villejuif 94801, France. Phone: 33 1 49 58 33 51. Fax: 33 1 49 58 33 48. E-mail: mchelbi@vjf.cnrs.fr.

light, ionizing radiation, or overexpression of PML IV only (6, 12, 13, 30, 31, 37).

A fascinating aspect of PML and p53 is that both are directly induced by type I IFN (8, 32, 43), and viruses from different families encode proteins which counteract their localization and/or activity (11, 15, 33). In addition, p53 was recently shown to be involved in antiviral defense (43). It remains to be determined, however, whether PML and p53 cooperate during viral infections and whether or not these interactions occur within PML NBs.

We describe herein the dialogue between PML and p53 during poliovirus infection and discuss the implications for the mechanisms underlying antiviral defense. We show that poliovirus infection induces PML phosphorylation through a mitogen-activated protein kinase (MAPK) pathway. Increased PML phosphorylation was associated with increased PML SUMOylation and p53 recruitment within the NBs, leading to p53 activation and induction of p53 target genes. This later effect, which requires the presence of PML, is transient as poliovirus induced p53 degradation in an MDM2- and proteasome-dependent manner. Taken together, our findings demonstrate the cross talk between PML and p53 during poliovirus infection and their cooperation in the antiviral defense.

MATERIALS AND METHODS

Cells and cell treatment. U2OS (derived from a human osteosarcoma) and U373MG (human glioblastoma astrocytoma) cells were grown at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. U373MG (transfected with the empty vector) cells or overexpressing PML III, U373MG-PML III cells were kept in medium supplemented with 0.5 mg of G418 (Gibco) per ml. The proteasome inhibitors epoxomicin (Calibiochem) and MG132 (carbobenzoxyl-leucinyl-leucinyl-leucinal-H), the caspase inhibitor, Z-VAD-fmk [*N*-benzyloxycarbonyl-Val-Ala-Asp (O-Me) fluoromethyl ketone], and MEK inhibitor UO126 (Cell Signaling Technology) were added to cells 1 h before infection and maintained during infection. MG132 and UO126 were used at a concentration of 10 μM, Z-VAD was used at a concentration of 100 μM, and epoxomicin was used at a concentration of 1 μM.

Virus stock preparation. Poliovirus (Sabin strain type 1) was amplified on HEp-2 cells, and viral titers were determined on these cells by measuring the 50% tissue culture infective dose ($TCID_{50}$) (24). After virus adsorption for 30 min at 37°C at the multiplicities of infection (MOI) indicated in the figure legends, cell monolayers were washed twice with phosphate-buffered saline (PBS) containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and incubated with Dulbecco's modified Eagle's medium.

Antibodies. Rabbit (H-238) anti-PML, mouse (PGM3) anti-PML, rabbit anti-SUMO-1 (FL-101), anti-p53 (DO1), and anti-MDM2 (N-20) antibodies were obtained from Santa Cruz. Anti-Sp100 was from H. Will. Rabbit anti-20S antibodies were from Affinity Research Products. Anti-ERK1/2 MAPK (where ERK is extracellular signal-regulated kinase), anti-phospho-ERK1/2 MAPK (Thr202/Tyr204), and anti-phospho-p53 (Ser15) antibodies were obtained from Cell Signaling Technology.

Immunofluorescence and confocal microscopy. Treated cells were fixed in 4% paraformaldehyde for 15 min at 4°C and permeabilized for 5 min with 0.1% Triton X-100 in PBS. They were then prepared for double immunofluorescence staining and analyzed by confocal microscopy. PML was detected with mouse or rabbit anti-PML antibodies at a dilution of 1/400 and the corresponding anti-immunoglobulin G (IgG) antibodies conjugated to Alexa 594. p53 was stained using DO1 antibodies at a dilution of 1/100 and the corresponding anti-mouse IgG antibody conjugated to Alexa 488 (Molecular Probe, Eugene, OR). Rabbit anti-Sp100, anti-SUMO-1, or anti-20S and the corresponding anti-rabbit IgG antibodies conjugated to Alexa 488 were used.

Nuclear matrix isolation. For in situ fractionation, cells grown on polylysine-coated coverslips were washed three times with ice-cold PBS. After being washed, the cells were sequentially extracted as previously described (17) with slight modifications: in fractionation experiments, 0.5% Triton X-100 was added in all digestion buffers instead of 1%. DNase treatment was done at room temperature. Monolayers of extracted cells at different steps of the nuclear matrix preparation were fixed as previously described and then processed for indirect immunofluorescence microscopy.

Western blot analysis of total cell extracts and RIPA soluble and insoluble fractions. For total cell extracts, cells were washed in PBS, lysed in hot Laemmli sample buffer, and boiled for 10 min. The radioimmunoprecipitation assay (RIPA) soluble fraction was obtained by lysing the equivalent of 10^6 cells in 50 μl of RIPA buffer (50 mM Tris, pH 7.5, 200 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 mM EDTA) for 20 min on ice, followed by centrifugation at 15,000 × *g* for 15 min to separate the RIPA soluble fraction from the pellet. This RIPA insoluble fraction was suspended in 50 μl of PBS and boiled for 10 min in 50 μl of 2× Laemmli buffer sample. Proteins were separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose membrane, blocked in 10% milk for 2 h, and then incubated overnight at 4°C with the first antibody and for 1 h with the appropriate peroxidase-coupled secondary antibodies (Amersham).

Apoptosis analysis. U2OS cells, transfected with unrelated, p53 small interfering RNA (siRNA) or PML siRNA, were noninfected or infected with poliovirus at an MOI 1 or 5 for 16 h or 30 h. Apoptotic cells were scored by terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assay with the APO-BrdU TUNEL assay kit (Molecular Probes, Eugene, OR).

Real-time quantitation mRNA. For *Mdm2* and *Noxa* mRNA quantification, cDNA synthesis was performed using oligo(dT) (Sigma, St. Louis, MO) and avian myeloblastosis virus reverse transcriptase as described by the manufacturer (Promega). Real-time quantitative PCR was performed using the FastStart DNA Master SYBR Green I kit and the LightCycler apparatus according to the protocol provided by the manufacturer (Roche Molecular Biochemicals Indianapolis, IN). PCR was carried out with the following oligonucleotide pairs: 5′-CGTGCCAAGCTTCTCTGTGA-3′ and 5′-GTCCGATGATCCTGCTGAT-3′ for Mdm2; 5′-AGTAGCTGGAAGTCGAGTGT-3′ and 5′-AGGTTCCTGAG CAGAAGAGT-3′ for Noxa; and 5′-AGCTCACTGGCATGGCCTTC-3′ and 5′-ACGCCTGCTTCACCACCTTC-3′ for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Results were normalized to GAPDH.

siRNA oligonucleotides. The oligonucleotides used in this study were purchased from Genset Oligos (Proligo, Boulder, CO). RNA duplexes 19 nucleotides long corresponding to PML, coding nucleotides 362 to 381 (common to all isoforms), or to p53, coding nucleotides 734 to 753, or to MDM2, coding nucleotides 482 to 501, were transfected into cells using Lipofectamine according to Invitrogen's procedures. After 24 h or 48 h, cells were infected by poliovirus. All the samples were analyzed 16 h later for apoptosis or viral replication, or cells were harvested and analyzed by Western blotting by using anti-p53, anti-PML, or anti-MDM2 antibodies.

RESULTS

Poliovirus infection induced the recruitment of PML from the nucleoplasm to the nuclear matrix, leading to the increase in PML NB size. We have previously shown that PML III isoform expression in the glioblastoma cell line U373MG confers resistance to VSV, influenza virus, and HFV (9) (34). Furthermore, infection with herpes simplex virus type 1 alters PML localization and expression (7). We tested the capacity of the PML III isoform to confer poliovirus resistance and that of poliovirus to alter PML localization and/or expression. U373MG and U373MG-PML III cells were infected with poliovirus at MOIs of 5, 1, and 0.1. Viral titers were determined at different times postinfection. Growth curves of poliovirus were similar in U373MG and U373MG-PML III cells, and the plateau was reached at 8 h postinfection. In addition, there were no differences in poliovirus-induced cytopathic effects in U373MG and U373MG-PML III cells (data not shown). Taken together, these results demonstrated that overexpression of PML III did not confer poliovirus resistance at an MOI of 5 (Fig. 1A), 1, or 0.1 (data not shown).

Immunofluorescence analysis performed from U373MG-PML III cells noninfected or infected with poliovirus for 4 h revealed that infection with poliovirus resulted in an increase in PML NB size where PML and SUMO-1 colocalized (Fig.

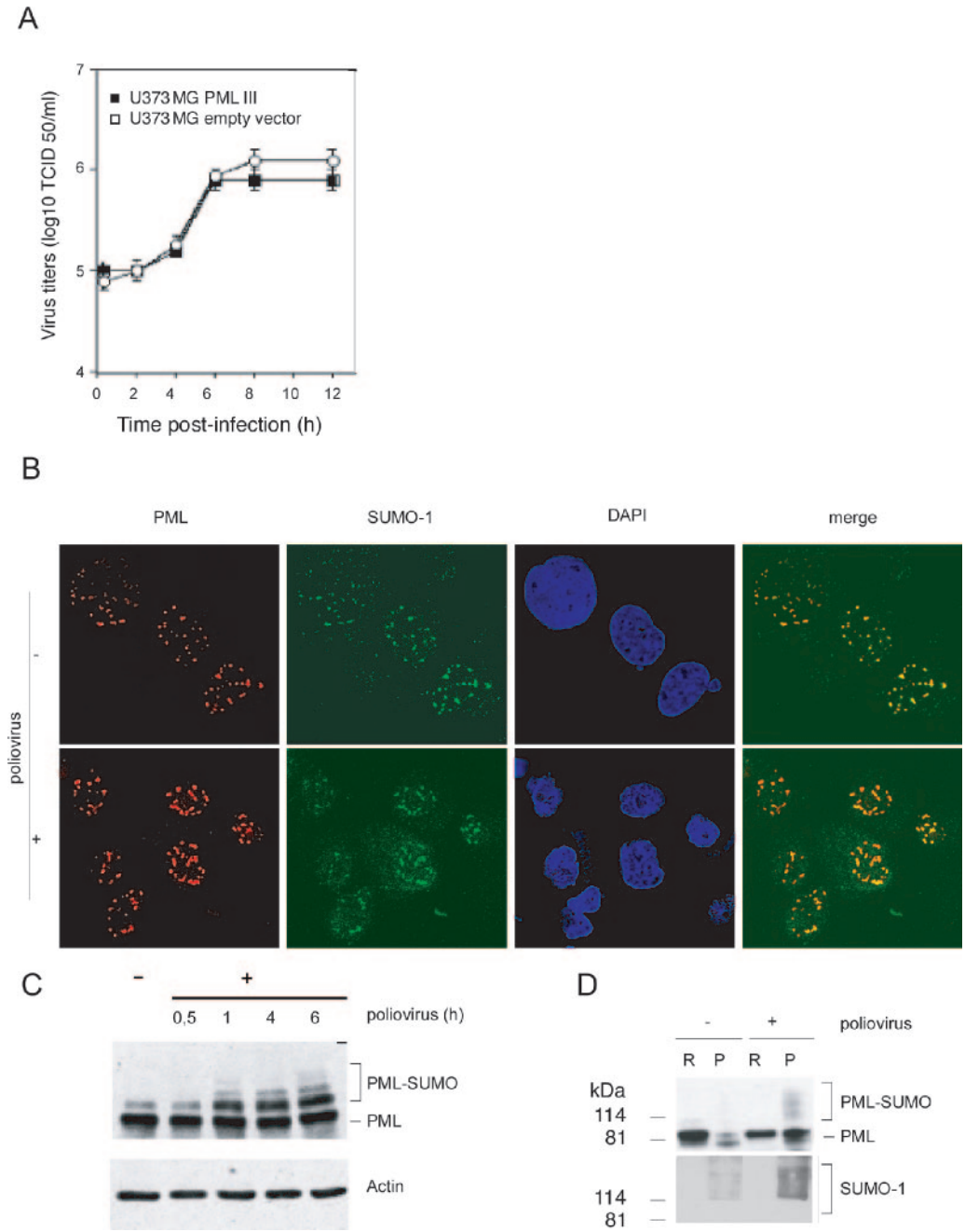

FIG. 1. (A) Overexpression of PML III isoform in U373MG cells did not confer resistance to poliovirus. U373MG expressing the empty vector and U373MG-PML III cells were infected with poliovirus at an MOI of 5. After viral adsorption for 30 min at 37°C, cells and supernatants were harvested at different times postinfection, and total virus yields were determined by $TCID_{50}$ assay. Each point represents the mean of three separate experiments. Error bars indicate the standard errors. (B) Confocal microscopy analysis of PML and SUMO-1 during poliovirus. U373MG-PML III cells were noninfected or infected with poliovirus at an MOI of 5 for 4 h. Double immunofluorescence staining was performed in noninfected and infected cells using monoclonal anti-PML antibodies visualized by Alexa 594 labeling and by rabbit anti-SUMO-1 antibodies and Alexa 488 labeling. (C) Fate of PML in poliovirus-infected cells. Cell extracts were prepared from infected U373MG-PML III cells with poliovirus at an MOI of 5 at different times. Twenty micrograms of protein extract of each sample was analyzed by Western blotting using anti-PML antibodies. (D) PML was recruited from the nucleoplasm (R) to the nuclear matrix (P) after poliovirus infection. The RIPA soluble (R) and the RIPA insoluble fractions (P), prepared from noninfected and infected cells at an MOI of 5 for 1 h, were analyzed by Western blotting by using anti-PML or anti-SUMO-1 antibodies.

1B). Thus, infection with the poliovirus alters PML NB localization.

In order to detect whether the expression of PML was also affected, extracts from U373MG-PML III cells infected with poliovirus during different periods were analyzed by Western blotting (Fig. 1C). That an equal amount of total protein was loaded is shown by the actin immunoblots. Two forms of PML having apparent molecular weights in SDS-PAGE of 80 and 95

were detected in U373MG-PML III cells. The higher, fainter, molecular species are known to represent the conjugated PML-SUMO forms (27, 47). Poliovirus infection increased PML conjugation to SUMO, the 95-kDa PML form became more abundant, and higher-molecular-weight forms were detected 1 h after infection and increased with the time of infection (Fig. 1C).

To determine whether poliovirus infection induced PML transfer from the nucleoplasm to the nuclear matrix, RIPA soluble and insoluble fractions from cells overexpressing PML III, noninfected or infected with poliovirus, were analyzed by Western blotting with anti-PML and anti-SUMO-1 antibodies. Poliovirus infection shifts PML toward the nuclear matrix where PML-SUMO-1 conjugated forms were detected (Fig. 1D). These results show that poliovirus infection induced PML conjugation to SUMO and the transfer of PML to the nuclear matrix, leading to the increase of PML NB size.

Phosphorylation of PML upon poliovirus infection is associated with enhanced PML SUMOylation. It is known that PML SUMOylation induced by As_2O_3 is dependent on a prior phosphorylation of PML (16). Therefore, we tested whether poliovirus could activate the MAPK ERK1/2. Analysis of extracts from U373MG cells infected during different short periods shows that phosphorylation of ERK1/2 was a transient and an early event; it was detected 15 min postinfection and reached a maximum level at between 30 and 45 min (Fig. 2A). Moreover, poliovirus-induced ERK1/2 activation was abolished in the presence of the pharmacologic MEK inhibitor UO126 (Fig. 2A).

CD155, the poliovirus receptor, has been implicated in the enhancement of serum- and platelet-derived growth factor-induced cell proliferation by activating the Ras-Raf-MEK-ERK signaling pathway (22). To confirm that poliovirus infection was involved in MAPK ERK1/2 activation, U373MG cells were incubated with a monoclonal antibody against CD155 for 1 h before poliovirus infection. Western blotting (Fig. 2B) and immunofluorescence analysis (data not shown) revealed that CD155 antibodies abolished poliovirus-induced ERK1/2 activation, demonstrating that poliovirus infection activated ERK1/2 signaling.

Therefore, we analyzed whether poliovirus infection could induce PML phosphorylation through the ERK pathway. U373MG cells were infected with poliovirus for 30 min in the absence or the presence of UO126. In noninfected cells, two PML forms were detected, a major form representing the nonphosphorylated PML and a minor form which disappeared when the extracts were treated with alkaline phosphatase (Fig. 2C). Poliovirus infection resulted in a shift in PML mobility in SDS-PAGE which could represent the phosphorylated form, as this form was not detected when the same extracts were treated with alkaline phosphatase or when cells were infected in the presence of UO126 (Fig. 2C). These results show that infection with poliovirus induced PML phosphorylation via MAPK ERK in the early step of infection.

To test whether inhibition of PML phosphorylation could alter its SUMOylation by poliovirus, U373MG-PML III cells were treated with UO126 before being infected by poliovirus for 5 h. Western blotting of cell extracts using anti-PML or anti-SUMO-1 antibodies demonstrated that the inhibitor of MAPK abrogated poliovirus-induced PML SUMOylation (Fig. 2D).

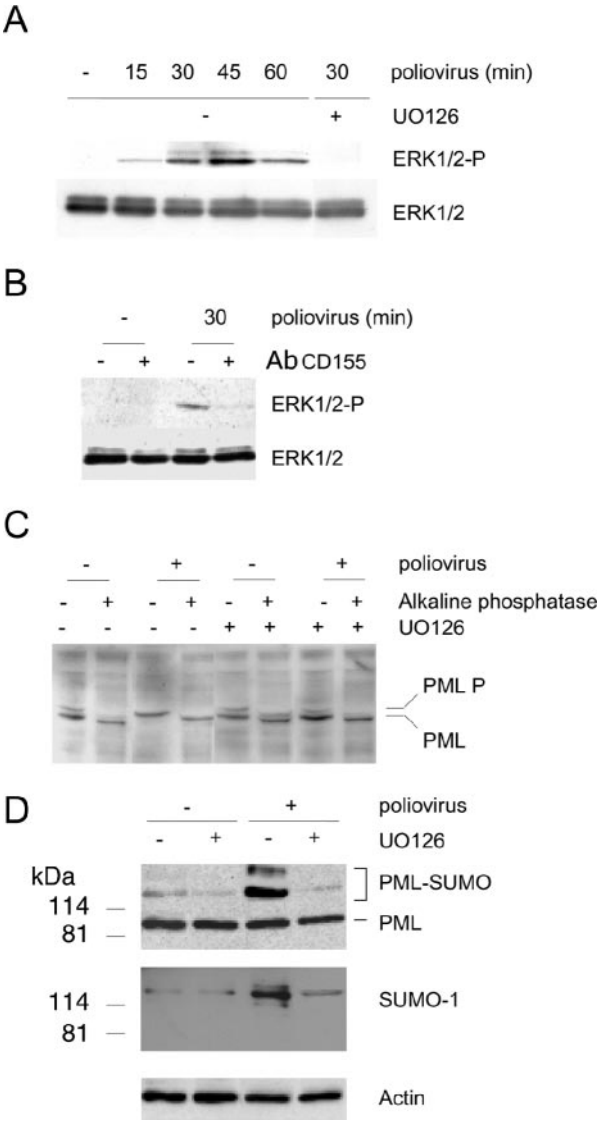

FIG. 2. (A) Poliovirus induced the MAPK ERK pathway. Cell extracts were prepared from U373MG cells infected for a short period in the absence or the presence of ERK inhibitor (UO126) and were analyzed by Western blotting using anti-ERK and anti-phospho ERK antibodies. (B) Anti-CD155 antibodies (Ab CD155) blocked poliovirus-induced ERK activation. U373MG cells were pretreated or not with anti-CD155 antibody for 1 h before infection with poliovirus at an MOI of 5 for 30 min. Western blotting was performed using anti-phospho ERK1/2 and anti-ERK1/2 antibodies. (C) Poliovirus induced PML phosphorylation. Cell extracts were prepared from U373MG cells noninfected or infected with poliovirus at an MOI of 5 for 30 min in the absence or the presence of UO126. Cell extracts were treated or not with alkaline phosphatase for 30 min at 37°C. All samples were analyzed by Western blotting by using anti-PML antibodies. (D) ERK inhibitor abolished poliovirus-induced PML SUMOylation. Cell extracts, prepared from U373MG-PML III cells noninfected or infected with poliovirus at an MOI of 5 for 1 h in the absence or the presence of UO126, were analyzed by Western blotting using anti-PML or anti-SUMO-1 antibodies.

Poliovirus infection induced the recruitment of Sp100 and p53 to PML NBs and a decrease in p53 expression only. The poliovirus-induced increase in PML NB size suggests the recruitment of cellular proteins in these organelles. Therefore,

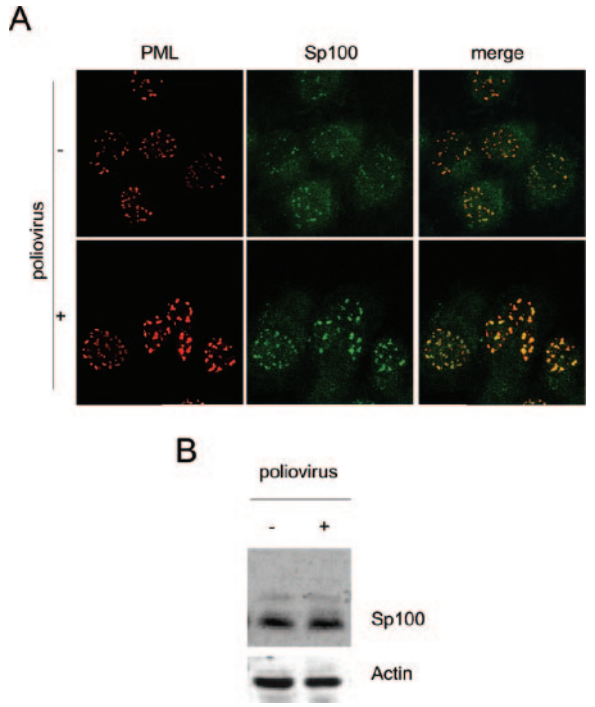

FIG. 3. Fate of Sp100 during poliovirus infection. U373MG-PML III cells were noninfected or infected with poliovirus at an MOI of 5 for 4 h. (A) Confocal microscopy analysis of PML and Sp100 in poliovirus-infected cells. Double immunofluorescence staining was performed using monoclonal anti-PML antibodies visualized by Alexa 594 labeling and by rabbit anti-Sp100 antibodies and Alexa 488 labeling. (B) Twenty micrograms of protein extract was analyzed by Western blotting using anti-Sp100 antibodies.

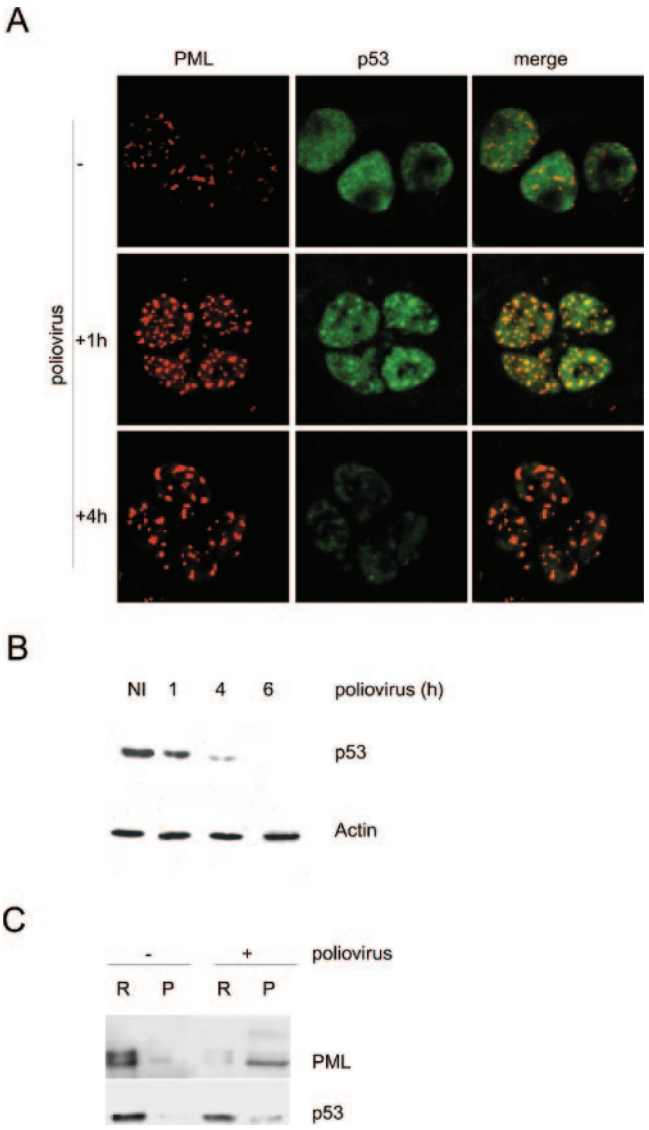

FIG. 4. (A) Confocal microscopy analysis of PML and p53 in poliovirus-infected cells. U373MG-PML III cells were noninfected or infected with poliovirus at an MOI of 5. Double immunofluorescence staining was performed at 1 h and 4 h later using rabbit anti-PML antibodies visualized by Alexa 594 labeling and by monoclonal anti-p53 antibodies and Alexa 488 labeling. (B) p53 expression was decreased during poliovirus infection. Twenty micrograms of protein extracts, prepared from U373MG-PML III cells infected for different times with poliovirus at an MOI of 5, was analyzed by Western blotting using anti-p53 or anti-actin antibodies. (C) p53 was recruited from the nucleoplasm (R) to the nuclear matrix (P) after poliovirus infection. The RIPA soluble (R) and the RIPA insoluble fractions (P), prepared from noninfected cells and cells infected for 1 h, were analyzed by Western blotting by using anti-PML or anti-p53 antibodies.

we tested the localization and the expression of other proteins known to be conjugated to SUMO-1 and associated to PML NBs, i.e., Sp100 and p53. Immunofluorescence (Fig. 3A) and Western blot (Fig. 3B) assays were performed from U373MG-PML III cells infected for 1 h and 4 h (Fig. 3A and data not shown). In noninfected cells, Sp100 was detected in PML NBs. After infection, Sp100 was further recruited and found to be colocalizing with PML in the large NBs.

As previously reported (41), in noninfected cells, two Sp100 forms were detected by Western blotting (Fig. 3B). None of these forms showed increased expression after poliovirus infection. Sp100 staining and expression were similar 1 h or 4 h postinfection (Fig. 3 and data not shown). These results show that poliovirus infection induced the recruitment of Sp100 to PML NBs without affecting its protein level.

In contrast, a clear difference was observed when these experiments were performed for p53 expression (Fig. 4A). In noninfected U373MG-PML III cells, p53 was found in the nucleus with very little colocalization with PML within the NBs. One hour postinfection, p53 was recruited to the NBs where it colocalized with PML. Four hours postinfection, PML labeling on the NBs was still strong, whereas that of p53 disappeared (Fig. 4A). A kinetics study showed clearly that poliovirus infection led to a decrease in the p53 level as early as 1 h and was no more detectable at 6 h postinfection (Fig. 4B).

The recruitment of p53 to PML NBs 1 h postinfection was further demonstrated by Western blot analysis of the RIPA soluble and insoluble fractions from noninfected and infected cells (Fig. 4C). One hour postinfection, both PML and p53 were shifted toward the nuclear matrix (assessed by the RIPA insoluble fraction) (Fig. 4C).

Taken together these data demonstrate that poliovirus induced the recruitment of Sp100 and p53 to PML NBs and diminished only the p53 protein level.

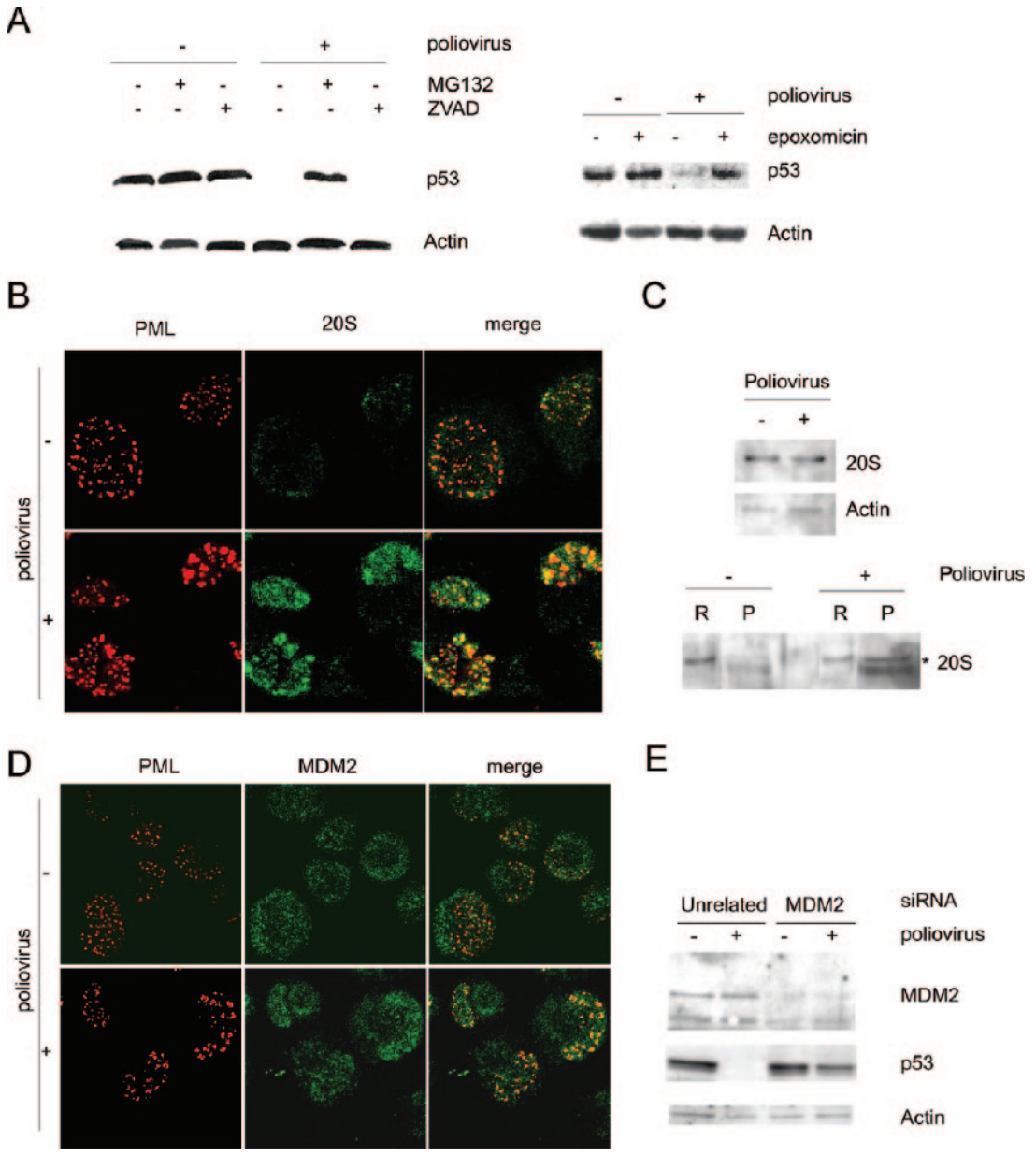

FIG. 5. Poliovirus induced p53 degradation in a proteasome- and MDM2-dependent manner. (A) The proteasome inhibitors (MG132 and epoxomicin) abrogated poliovirus-induced p53 degradation. Cell extracts were prepared from U373MG-PML III cells noninfected or infected during 4 h with poliovirus at an MOI of 5 in the absence or the presence of MG132, epoxomicin, or Z-VAD-fmk. Twenty micrograms of protein extract of each sample was analyzed by Western blotting using anti-p53 or anti-actin antibodies. (B) Confocal microscopy analysis of PML and 20S in poliovirus-infected cells. U373MG-PML III cells were noninfected or infected with poliovirus at an MOI of 5 for 1 h. Double immunofluorescence staining was performed using monoclonal anti-PML antibodies visualized by Alexa 594 and with rabbit anti-20S antibodies and Alexa 488 labeling. (C) Cells were treated as in described in panel B, and total cell extracts (upper panel) or RIPA soluble (R) and RIPA insoluble fractions (P) (lower panel) were analyzed by Western blotting using anti-20S antibodies. (D) Confocal microscopy analysis of PML and MDM2 in poliovirus-infected cells. U373MG-PML III cells were noninfected or infected with poliovirus at an MOI of 5 for 1 h. Double immunofluorescence staining was performed using monoclonal anti-PML antibodies visualized by Alexa 594 and rabbit anti-MDM2 antibodies followed by Alexa 488 labeling. (E) Poliovirus-induced p53 degradation required MDM2. U373MG-PML III cells were transfected with unrelated siRNA or MDM2-specific siRNA. One day later, cells were infected with poliovirus for 6 h at an MOI of 5, and cell extracts were analyzed by Western blotting using anti-p53, anti-MDM2, or anti-actin antibodies.

The proteasome inhibitors, MG132 or epoxomicin, abrogated poliovirus-induced p53 degradation. To test the implication of the proteasome or the caspase pathways in poliovirus-induced p53 decrease, U373MG-PML III cells were infected with poliovirus both in the absence and in the presence of MG132, epoxomicin, or Z-VAD. Addition of MG132 or epoxomicin, but not of Z-VAD, abrogated poliovirus-induced p53 degradation in cells overexpressing PML III (Fig. 5A). Then, we analyzed whether the localization of the 20S proteasome component was modified after poliovirus infection

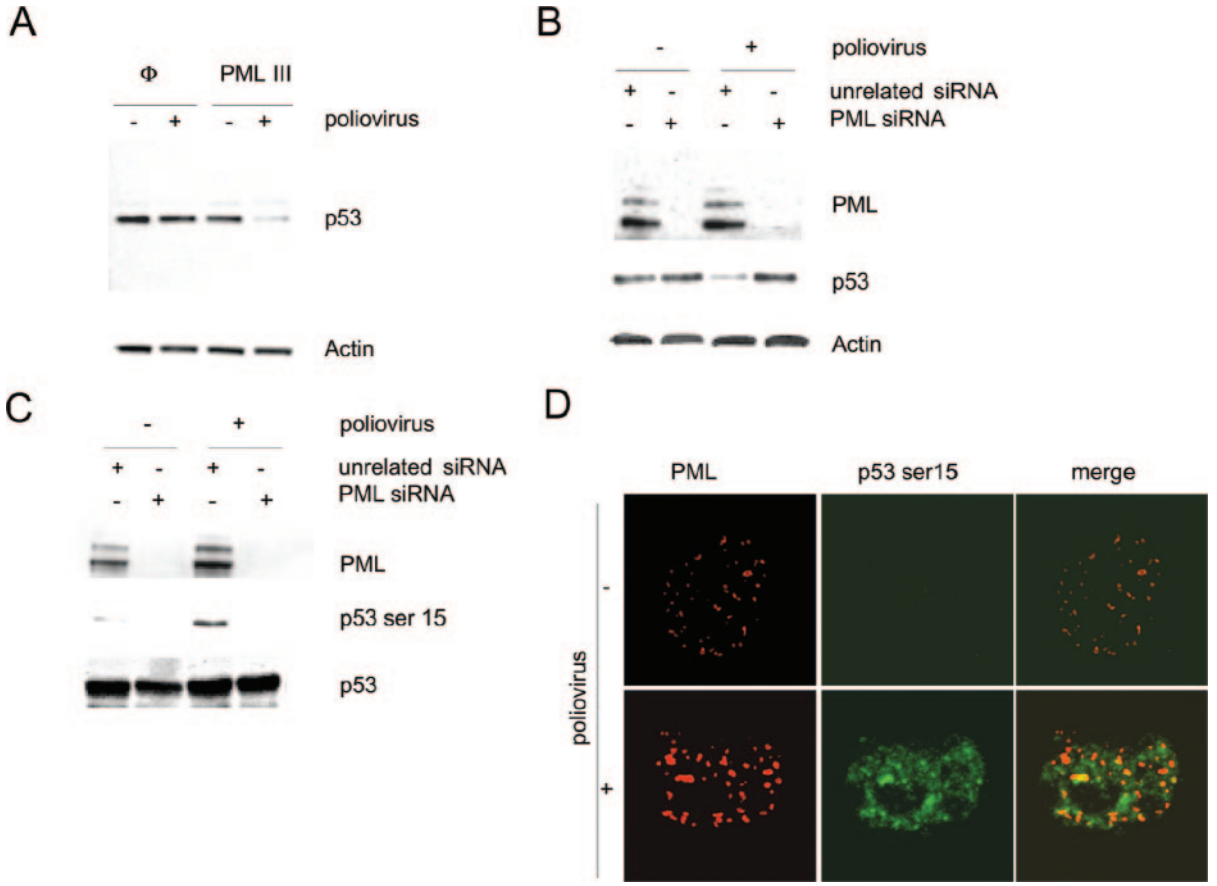

FIG. 6. Poliovirus induced p53 degradation in a PML III-dependent manner. (A) Poliovirus-induced p53 degradation was accelerated by PML III expression. Twenty micrograms of protein extracts, prepared from U373MG and U373MG-PML III cells infected for 4 h with poliovirus at an MOI of 5, was analyzed by Western blotting using anti-p53 and anti-actin antibodies. (B) Knock-down of PML in U373MG-PML III cells delayed poliovirus-induced p53 degradation. U373MG-PML III cells were transfected with PML siRNA. Two days later, cells were left noninfected or were infected with poliovirus for 4 h at an MOI of 5. Cell extracts were analyzed by Western blotting with anti-PML, anti-p53, or anti-actin antibodies. (C) Knock-down of PML in U373MG-PML III cells abolished poliovirus-induced p53 phosphorylation. U373MG-PML III cells were transfected with PML siRNA. Two days later, cells were infected with poliovirus for 1 h at an MOI of 5. Cell extracts were analyzed by Western blotting with anti-PML or anti-phospho Ser15 p53 antibodies. (D) Phosphorylated p53 (Ser15) was found colocalizing with PML within the NBs. Double immunofluorescence staining using anti-PML and anti-p53Ser15 antibodies was performed from nuclear matrix isolated from cells infected with poliovirus at an MOI of 5 for 1 h. Confocal microscopy analysis is shown.

(Fig. 5B). In noninfected U373MG-PML III cells, the 20S labeling was weak in the nucleus. Upon infection, the 20S protein was more abundant in the nucleus and presented a punctuate labeling colocalizing with PML NBs (Fig. 5B). Total cell extracts (Fig. 5C, upper panel) as well as RIPA soluble and insoluble fractions (Fig. 5C, lower panel) from noninfected and infected cells demonstrated that poliovirus infection did not increase the expression of 20S but induced its transfer to the nuclear matrix (Fig. 5C).

Knock-down of MDM2 expression partially blocked p53 degradation induced by poliovirus infection. We investigated whether MDM2 is implicated in poliovirus-induced p53 degradation. Double immunofluorescence analysis for MDM2 and PML revealed that at 1 h postinfection poliovirus induced the recruitment of MDM2 within the PML NBs, where both proteins colocalized (Fig. 5D). Then, we tested the effect of the knock-down of MDM2 on the decrease of p53 expression in poliovirus-infected cells. U373MG-PML III cells, transfected with unrelated siRNA or MDM2-specific siRNA, were infected for 4 h, and cell extracts were analyzed by Western blotting for MDM2 and p53 expression (Fig. 5E). Poliovirus infection induced p53 degradation in the presence of unrelated siRNA. In contrast, less degradation was observed when MDM2 expression was down-regulated (Fig. 5E). Taken together these results show that poliovirus infection induced p53 degradation in an MDM2- and proteasome-dependent manner.

Expression of PML III accelerated poliovirus-induced p53 degradation. From experiments shown in Fig. 4B, it appeared that in U373MG-PML III cells, the p53 protein level decreased at 1 h postinfection and was not detectable 6 h postinfection. To determine whether PML expression could accelerate poliovirus-induced p53 degradation, we analyzed the fate of p53 in U373MG cells infected for different times with poliovirus. In infected U373MG cells, the decrease of p53 protein level was only observed at 8 h postinfection (data not shown). In order to confirm this result, we analyzed extracts from U373MG and U373MG-PML III cells, noninfected or infected with poliovirus for 4 h. The results presented in Fig. 6A show that the p53

protein level was decreased in U373MG-PML III cells and not in U373MG cells, suggesting that overexpression of PML III accelerated poliovirus-induced p53 degradation. To confirm this result, we used siRNA PML to down-regulate PML expression in U373MG-PML III cells (Fig. 6B). U373MG-PML III cells transfected with siRNA PML were noninfected or infected with poliovirus for 4 h, and cell extracts were analyzed by Western blotting for PML and p53 expression. Down-regulation of PML expression was accompanied by an abrogation of poliovirus-induced p53 degradation (Fig. 6B). Taken together these findings demonstrate that the expression of PML III accelerated poliovirus-induced p53 degradation.

PML was necessary for poliovirus-induced p53 Ser15 phosphorylation. To test whether poliovirus induced p53 phosphorylation and whether this involved PML, U373MG-PML III cells transfected with unrelated or specific PML siRNA were infected for 1 h and analyzed by Western blotting with antiphospho p53Ser15 and anti-PML antibodies (Fig. 6C). Poliovirus infection induced Ser15 p53 phosphorylation, which was not observed when PML expression was down-regulated by siRNA, suggesting that this modification occurred in a PML-dependent manner. Interestingly, when double immunofluorescence staining using anti-PML and anti-p53 Ser15 antibodies was performed on a nuclear matrix isolated from cells infected under the same conditions, we could observe a colocalization in many dots between PML and phosphorylated p53 (Fig. 6D). Together, these results suggest that PML NBs could be nuclear organelles in which p53 phosphorylation and degradation take place after poliovirus infection.

Implication of p53 in poliovirus replication and poliovirus-induced apoptosis. In U373MG cells, the endogenous *p53* gene is mutated at codon 273 (CGT-CAT; Arg-His). This mutation does not lead to conformational change but completely abrogates specific DNA binding and transcriptional activity of the p53 protein (35).

To test whether poliovirus-induced p53 phosphorylation could increase p53 target genes and whether this is dependent on PML expression, we used a cell line expressing a wild-type p53, the osteosarcoma U2OS cells. Kinetics of virus replication in U2OS cells infected with poliovirus at an MOI of 1 or 5 showed that the plateau was reached at 24 h postinfection (Fig. 7A and data not shown). We verified that poliovirus induced p53 degradation in U2OS expressing wild-type p53. In these cells p53 degradation was observed at 16 h postinfection, corresponding to the exponential phase of poliovirus replication (Fig. 7B).

We then investigated whether poliovirus infection could increase the transcriptional activity of p53. For this, we measured the level of two p53 target genes (*Mdm2* and *Noxa*) by quantitative reverse transcription-PCR in U2OS cells at 6 h postinfection (Fig. 7C). Also, we tested the contribution of PML in the U2OS cells both by down-regulating its expression by siRNA and by transfecting these cells with PML III-expressing vector. Infection with poliovirus induced an increase in *Mdm2* and *Noxa* levels. This increase was augmented by PML III expression. In contrast, poliovirus did not alter *Mdm2* and *Noxa* expression when endogenous PML expression was down-regulated by siRNA (Fig. 7C).

These findings highlight the importance of the cross talk between PML and p53 during poliovirus infection, leading to p53 recruitment within PML NBs, p53 phosphorylation, and activation of p53 target genes.

Effect of p53 on poliovirus-induced apoptosis and viral replication. To determine the biological significance of the activation of gene expression by p53 during infection, we investigated the role of p53 and PML in poliovirus-induced apoptosis in cells expressing wild-type p53. U2OS cells, transfected with unrelated siRNA, p53 siRNA, or PML siRNA were infected with poliovirus at an MOI of 5 for 16 h or 30 h. By Western blotting we checked the knock-down of endogenous p53 or PML in U2OS cells by specific siRNA (Fig. 7D). We performed a TUNEL assay to test apoptosis (Fig. 7E). Apoptosis induced in U2OS cells 16 h postinfection at an MOI of either 1 or 5 was decreased when p53 or PML was down-regulated by siRNA. This effect was transient and was not observed at 30 h postinfection. This effect was expected since p53 was degraded 16 h postinfection (Fig. 7B).

We next investigated whether p53 or PML altered poliovirus replication. Detailed kinetics of virus production with p53 siRNA in U2OS cells revealed that the highest differences in viral replication were observed at 16 h postinfection (data not shown). Consequently, we performed the experiments to determine the effects of p53 siRNA or PML siRNA knock-down on poliovirus replication at 16 h postinfection.

U2OS cells were transfected with unrelated siRNA, specific p53 siRNA, or PML siRNA and were infected with poliovirus at an MOI of 0.1 or 1. Virus yield was determined at 16 h postinfection (Fig. 7F). Knock-down of p53 or PML resulted in higher poliovirus replication, demonstrating the importance of the cross talk between p53 and PML in antiviral defense during poliovirus infection.

Taking into consideration that siRNA PML abolished both poliovirus-induced *p53* gene expression and poliovirus-induced p53-dependent apoptosis, our results suggest that counteraction of poliovirus replication by p53 was dependent on PML expression.

DISCUSSION

We have presented results demonstrating that poliovirus infection induced PML phosphorylation, which was associated with increased PML SUMOylation, its transfer from the nucleoplasm to the nuclear matrix, and the recruitment of p53, 20S, and MDM2 to the PML NBs. PML phosphorylation, the first step of PML modification, occurred very rapidly within 30 min after poliovirus infection and was dependent on ERK activation. PML SUMOylation did not occur following infection when cells were treated with the MEK1/2 inhibitor UO126, suggesting that poliovirus-induced PML phosphorylation preceded PML SUMOylation. How ERK-phosphorylated PML leads to higher PML SUMOylation is unknown. It has been reported that phosphorylation of PML by MAPKs plays an important role in the control of PML-dependent apoptosis in response to As_2O_3 (16). This modification may regulate PML traffic from the nucleoplasm to the nuclear matrix where PML SUMOylation is believed to occur (27, 47).

Multiple isoforms of PML (I to VII) have been identified (21), most of which are found in the nucleus. The biological significance of the different PML isoforms in mammalian cells has not been well characterized. It has been reported that p53

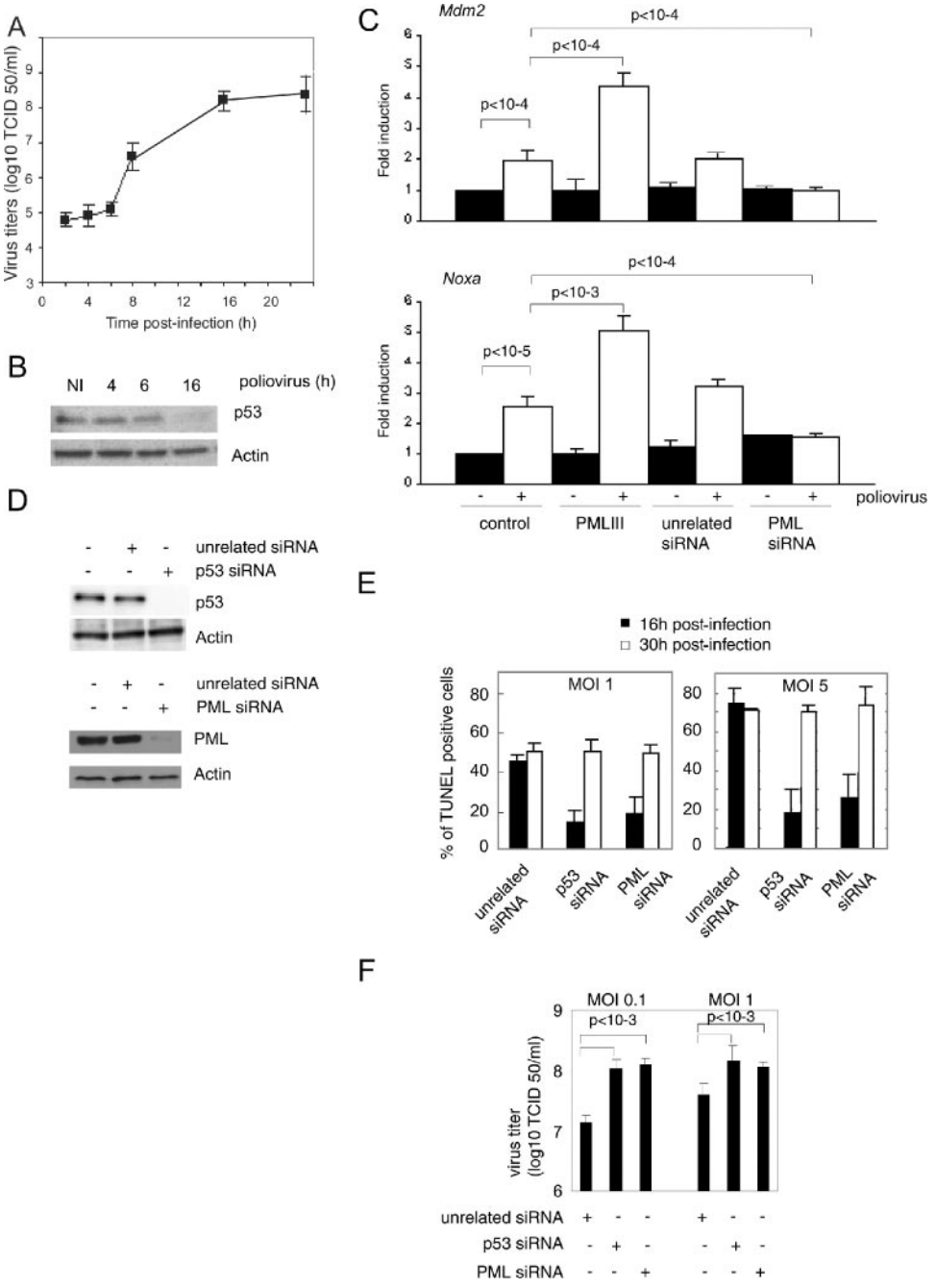

FIG. 7. (A) U2OS cells were infected with poliovirus at an MOI of 5. Cells and supernatants were harvested at different times postinfection, and total virus yields were determined by a $TCID_{50}$ assay. Each point represents the mean of three separate experiments. (B) Kinetics of p53 decrease in U2OS cells infected with poliovirus. Extracts from cells infected with poliovirus at an MOI of 5 for different periods were analyzed by Western blotting for p53 expression. (C) Poliovirus infection induced p53 gene activation. U2OS transfected with unrelated siRNA, PML siRNA, or PML III-expressing vector were noninfected or infected with poliovirus for 6 h at an MOI of 5. RNA was extracted in all samples, and *Mdm2* and *Noxa* expression were tested by the real-time PCR method. Results were normalized to GAPDH. The average and standard deviations of three independent assays are shown; the statistical significance (*P* values) was calculated using a Student's *t* test. (D) Knock-down of p53 or PML in U2OS cells by specific siRNA is shown by Western blotting. (E) Poliovirus infection induced apoptosis in a p53- and PML-dependent manner. U2OS cells were transfected with unrelated siRNA, p53 siRNA, or PML siRNA. After 24 h, cells were infected with poliovirus for 16 h or 30 h at an MOI of 1 or 5. The percentages of apoptotic cells were scored by TUNEL assay; the average and standard deviations of three independent experiments are shown. (F) Poliovirus replication was higher in the absence of p53 or PML. U2OS cells were transfected with unrelated siRNA, p53 siRNA, or PML siRNA. After 24 h cells were infected with poliovirus at an MOI of 0.1 or 1. Cells and supernatants were harvested at 16 h postinfection, and total virus yields were determined by $TCID_{50}$ assay. Each point represents the mean of three separate experiments. Error bars indicate the standard errors of the means. The statistical significance (*P* values) was calculated using a Student's *t* test.

interacts directly with the carboxy terminal end of PML IV only (13). We found that in noninfected cells, only the PML IV isoform strongly recruited p53, but in sharp contrast, all PML isoforms tested (II, III, IV, and V) highly recruited p53 to NBs in poliovirus-infected cells (data not shown).

We have shown previously that overexpression of PML III in U373MG cells inhibits the replication of VSV, influenza virus, and HFV (9, 34). The capacity of PML III to inhibit these viruses was observed in U373MG cells, where an endogenous *p53* gene mutation abrogates specific DNA binding and transcriptional activity of p53 (35), demonstrating that PML III possesses p53-independent antiviral activities.

We demonstrate in this report that PML III expression did not confer poliovirus resistance in p53-inactivated cells even though poliovirus induced p53Ser15 phosphorylation in PML-dependent manner.

Interestingly, in p53 wild-type cells, infection by poliovirus induced, in a PML-dependent way, activation of p53 target genes, *Mdm2* and *Noxa*, leading to the induction of apoptosis in poliovirus-infected cells and to the inhibition of viral replication. All of these effects were abolished when endogenous PML expression was down-regulated by siRNA. In contrast, these effects were augmented by PML III expression. In addition, knock-down of p53 by siRNA resulted in higher poliovirus replication in U2OS cells.

In several aspects PML and p53 seem to be linked. Both are directly induced by type I IFN (8, 32, 40, 43), and viruses from different families encode proteins that alter PML and/or p53 through different means, i.e., localization, expression, stability, or activity (11, 15, 33). The p53 protein is recruited within PML NBs upon Ras activation or after exposure to UV light or ionizing radiation (6, 12, 30, 31, 37). Overexpression of PML IV equally recruits p53 and increases its transcriptional activity (13). Finally, it was recently reported that p53, like PML (4, 9, 33, 34), is involved in the antiviral defense, as $p53^{-/-}$ mice have a much higher death rate after VSV infection than do wild-type mice (43). Furthermore, virus titers in the serum are also much higher in p53-deficient mice than in normal mice (43). Here, we demonstrate that p53 does not possess intrinsic and independent antiviral properties. Indeed, poliovirus regulates PML and NBs which, in turn, affects p53 activity.

PML and PML NBs are implicated in apoptosis (2, 19, 42) and antiviral responses (33). This may explain why PML NBs are preferential targets of viral infection. A central question is whether, during viral infection, PML NBs themselves are sites of specific activity that PML proteins do not exhibit elsewhere in the nucleoplasm. Taken together, our results show the mechanisms by which p53 and PML cooperate in elaborating a cellular antiviral response.

Many viruses develop strategies to inhibit apoptosis in infected cells (1, 46). Very recently, it has been reported that hepatitis C virus core protein inhibits p53-target gene expression by targeting PML IV (18). We have demonstrated that poliovirus counteracted this cellular defense by inducing p53 degradation on PML NBs in a proteasome- and MDM2-dependent manner. The degradation of p53 was accelerated in cells overexpressing PML III. In contrast, down-regulation by siRNA of PML expression delayed poliovirus-induced p53 degradation. Understanding the cross talk between PML and p53 during viral infection is crucial to elucidate how viruses alter components of the apoptotic pathway, counteracting or participating in the antiviral defense.

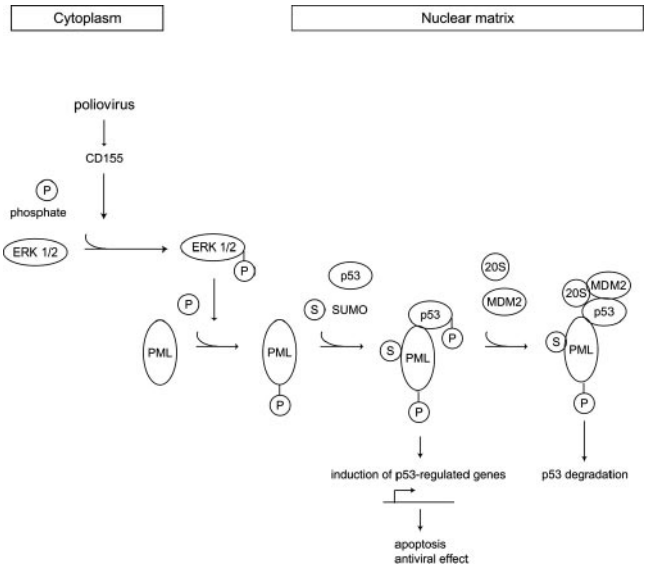

FIG. 8. Cross talk between PML and p53 during poliovirus infection.

Our results demonstrate in poliovirus-infected cells the importance of PML in recruiting p53 within the NBs, leading to its phosphorylation on serine 15 and to the activation of its target genes. Taken together, these findings show that PML NBs could be nuclear organelles where p53 recruitment, phosphorylation, activation of target genes, and degradation take place during poliovirus infection.

Collectively, our findings lead to the following conclusions presented in the model illustrated in Fig. 8.(i) Poliovirus infection induced ERK activation as early as 15 min postinfection, leading to PML phosphorylation, detected 30 min postinfection; (ii) this phosphorylation was necessary for poliovirus-induced PML SUMOylation, observed 1 h postinfection; (iii) poliovirus shifted PML from the nucleoplasm to the nuclear matrix, leading to the recruitment of p53 to PML NBs; (iv) poliovirus induced, in a PML-dependent manner, p53 phosphorylation and p53 target genes, leading to apoptosis in infected cells and inhibition of viral replication; (v) poliovirus counteracted p53 by inducing both the recruitment of 20S and MDM2 to PML NBs and p53 degradation in an MDM2- and proteasome-dependent manner.

Viruses need to multiply in the infected host and have evolved different strategies to impair the cellular antiviral response. Our results provide evidence of how poliovirus counteracts p53 antiviral activity by regulating PML and NBs, thus leading to p53 degradation.

ACKNOWLEDGMENTS

We acknowledge Hans Will for the gift of anti-Sp100 antibodies, Akio Nomoto for the gift of anti-CD155 antibodies, and Evelyne May for fruitful discussion. We thank Frédéric Galisson for technical assistance.

This work was supported by grants from the Centre Nationale National pour la Recherche Scientifique and Agence Nationale de la Recherche sur le Sida.

REFERENCES

1. **Barber, G. N.** 2001. Host defense, viruses and apoptosis. Cell Death Differ. **8:**113–126.
2. **Bernardi, R., and P. P. Pandolfi.** 2003. Role of PML and the PML-nuclear body in the control of programmed cell death. Oncogene **22:**9048–9057.
3. **Bodian, D.** 1955. Emerging concept of poliomyelitis infection. Science **122:** 105–108.
4. **Bonilla, W. V., D. D. Pinschewer, P. klenerman, V. Rousson, M. Gaboli, P. Pandolfi, R. M. Zinkernagel, M. S. Salvato, and H. Hengartner.** 2002. Effects of promyelocytic leukemia protein on virus-host balance. J. Virol. **76:**3810–3818.
5. **Buenz, E. J., and C. L. Howe.** 2005. Picornaviruses and cell death. Trends Microbiol.
6. **Carbone, R., M. Pearson, S. Minucci, and P. G. Pelicci.** 2002. PML NBs associate with the hMre11 complex and p53 at sites of irradiation induced DNA damage. Oncogene **21:**1633–1640.
7. **Chelbi-Alix, M. K., and H. de The.** 1999. Herpes virus induces proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100. Oncogene **18:**935–941.
8. **Chelbi-Alix, M. K., L. Pelicano, F. Quignon, M. H. M. Koken, L. Venturini, M. Stadler, J. Pavlovic, L. Degos, and H. de Thé.** 1995. Induction of the PML protein by interferons in normal and APL cells. Leukemia **9:**2027–2033.
9. **Chelbi-Alix, M. K., F. Quignon, L. Pelicano, M. H. M. Koken, and H. de The.** 1998. Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein. J. Virol. **72:**1043–1051.
10. **Couderc, T., C. Christodoulou, H. Kopecka, S. Marsden, L. F. Taffs, R. Crainic, and F. Horaud.** 1989. Molecular pathogenesis of neural lesions induced by poliovirus type 1. J. Gen. Virol. **70:**2907–2918.
11. **Everett, R. D.** 2001. DNA viruses and viral proteins that interact with PML nuclear bodies. Oncogene **20:**7266–7273.
12. **Ferbeyre, G., E. de Stanchina, E. Querido, N. Baptiste, C. Prives, and S. W. Lowe.** 2000. PML is induced by oncogenic *ras* and promotes premature senescence. Genes Dev. **14:**2015–2027.
13. **Fogal, V., M. Gostissa, P. Sandy, P. Zacchi, T. Sternsdorf, K. Jensen, P. P. Pandolfi, H. Will, C. Schneider, and G. Del Sal.** 2000. Regulation of p53 activity in nuclear bodies by a specific PML isoform. EMBO J. **19:**6185–6195.
14. **Girard, S., T. Couderc, J. Destombes, D. Thiesson, F. Delpeyroux, and B. Blondel.** 1999. Poliovirus induces apoptosis in the mouse central nervous system. J. Virol. **73:**6066–6072.
15. **Gostissa, M., T. G. Hofmann, H. Will, and G. Del Sal.** 2003. Regulation of p53 functions: let's meet at the nuclear bodies. Curr. Opin. Cell Biol. **15:** 351–357.
16. **Hayakawa, F., and M. L. Privalsky.** 2004. Phosphorylation of PML by mitogen-activated protein kinases plays a key role in arsenic trioxide-mediated apoptosis. Cancer Cell **5:**389–401.
17. **He, D. C., J. A. Nickerson, and S. Penman.** 1990. Core filaments of the nuclear matrix. J. Cell Biol. **110:**569–580.
18. **Herzer, K., S. Weyer, P. H. Krammer, P. R. Galle, and T. G. Hofmann.** 2005. Hepatitis C virus core protein inhibits tumor suppressor protein promyelocytic leukemia function in human hepatoma cells. Cancer Res. **65:**10830–10837.
19. **Hofmann, T. G., and H. Will.** 2003. Body language: the function of PML nuclear bodies in apoptosis regulation. Cell Death Differ. **10:**1290–1299.
20. **Ishov, A., A. Sotnikov, D. Negorev, O. Vladimirova, N. Neff, T. Kamitani, E. Yeh, J. Strauss III, and G. Maul.** 1999. PML is critical for ND10 formation and recruits the PML-interacting protein Daxx to this nuclear structure when modified by SUMO-1. J. Cell Biol. **147:**221–223.
21. **Jensen, K., C. Shiels, and P. S. Freemont.** 2001. PML protein isoforms and the RBCC/TRIM motif. Oncogene **20:**7223–7233.
22. **Kakunaga, S., W. Ikeda, T. Shingai, T. Fujito, A. Yamada, Y. Minami, T. Imai, and Y. Takai.** 2004. Enhancement of serum- and platelet-derived growth factor-induced cell proliferation by Necl-5/Tage4/poliovirus receptor/CD155 through the Ras-Raf-MEK-ERK signaling. J. Biol. Chem. **279:**36419–36425.
23. **Lallemand-Breitenbach, V., J. Zhu, F. Puvion, M. Koken, N. Honore, A. Doubeikovsky, E. Duprez, P. P. Pandolfi, E. Puvion, P. Freemont, and H. de The.** 2001. Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11S proteasome recruitment, and As2O3-induced PML or PML/retinoic acid receptor alpha degradation. J. Exp. Med. **193:**1361–1371.
24. **Melnick, J. L., H. A. Wenner, and C. A. Phillips.** 1979. Enteroviruses, p. 471–534. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial, and chlamydial infections, 5th ed. American Public Health Assoc., Washington, D.C.
25. **Muller, S., and A. Dejean.** 1999. Viral immediate-early proteins abrogate the modification by SUMO-1 of PML and Sp100 proteins, correlating with nuclear body disruption. J. Virol. **73:**5137–5143.
26. **Muller, S., M. J. Matunis, and A. Dejean.** 1998. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J. **17:**61–70.
27. **Muller, S., W. H. Miller, and A. Dejean.** 1998. Trivalent antimonials induce degradation of the PML-RAR oncoprotein and reorganization of the promyelocytic leukemia nuclear bodies in acute promyelocytic leukemia NB4 cells. Blood **92:**4308–4316.
28. **Negorev, D., and G. G. Maul.** 2001. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. Oncogene **20:**7234–7242.
29. **Neznanov, N., K. M. Chumakov, L. Neznanova, A. Almasan, A. K. Banerjee, and A. V. Gudkov.** 2005. Proteolytic cleavage of the p65-RelA subunit of NF-κB during poliovirus infection. J. Biol. Chem. **280:**24153–24158.
30. **Pearson, M., R. Carbone, C. Sebastiani, M. Cioce, M. Fagioli, S. Saito, Y. Higashimoto, E. Appella, S. Minucci, P. P. Pandolfi, and P. G. Pelicci.** 2000. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature **406:**207–210.
31. **Pearson, M., and P. G. Pelicci.** 2001. PML interaction with p53 and its role in apoptosis and replicative senescence. Oncogene **20:**7250–7256.
32. **Porta, C., R. Hadj-Slimane, M. Nejmeddine, M. Pampin, M. G. Tovey, L. Espert, S. Alvarez, and M. K. Chelbi-Alix.** 2005. Interferons alpha and gamma induce p53-dependent and p53-independent apoptosis, respectively. Oncogene **24:**605–615.
33. **Regad, T., and M. K. Chelbi-Alix.** 2001. Role and fate of PML nuclear bodies in response to interferon and viral infections. Oncogene **20:**7274–7286.
34. **Regad, T., A. Saib, V. Lallemand-Breitenbach, P. P. Pondolfi, H. de The, and M. K. Chelbi-Alix.** 2001. PML mediates the interferon-induced antiviral state against a complex retrovirus via its association with the viral transactivator. EMBO. J. **20:**3495–3505.
35. **Rolley, N., S. Butcher, and J. Milner.** 1995. Specific DNA binding by different classes of human p53 mutants. Oncogene **11:**763–770.
36. **Romanova, L. I., G. A. Belov, P. V. Lidsky, E. A. Tolskaya, M. S. Kolesnikova, A. G. Evstafieva, A. B. Vartapetian, D. Egger, K. Bienz, and V. I. Agol.** 2005. Variability in apoptotic response to poliovirus infection. Virology **331:**292–306.
37. **Salomoni, P., and P. P. Pandolfi.** 2002. The role of PML in tumor suppression. Cell **108:**165–170.
38. **Seeler, J. S., and A. Dejean.** 2001. SUMO: of branched proteins and nuclear bodies. Oncogene **20:**7243–7249.
39. **Sharma, R., S. Raychaudhuri, and A. Dasgupta.** 2004. Nuclear entry of poliovirus protease-polymerase precursor 3CD: implications for host cell transcription shut-off. Virology **320:**195–205.
40. **Stadler, M., M. K. Chelbi-Alix, M. H. M. Koken, L. Venturini, C. Lee, A. Saïb, F. Quignon, L. Pelicano, M.-C. Guillemin, C. Schindler, and H. de Thé.** 1995. Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. Oncogene **11:**2565–2573.
41. **Sternsdorf, T., K. Jensen, and H. Will.** 1997. Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. J. Cell Biol. **139:**1621–1634.
42. **Takahashi, Y., V. Lallemand-Breitenbach, J. Zhu, and H. de The.** 2004. PML nuclear bodies and apoptosis. Oncogene **23:**2819–2824.
43. **Takaoka, A., S. Hayakawa, H. Yanai, D. Stoiber, H. Negishi, H. Kikuchi, S. Sasaki, K. Imai, T. Shibue, K. Honda, and T. Taniguchi.** 2003. Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. Nature **424:**516–523.
44. **Wang, Z.-G., D. Ruggero, S. Ronchetti, S. Zhong, M. Gaboli, R. Rivi, and P. P. Pandolfi.** 1998. PML is essential for multiple apoptotic pathways. Nat. Genet. **20:**266–272.
45. **Weidman, M. K., P. Yalamanchili, B. Ng, W. Tsai, and A. Dasgupta.** 2001. Poliovirus 3C protease-mediated degradation of transcriptional activator p53 requires a cellular activity. Virology **291:**260–271.
46. **Whitton, J. L., C. T. Cornell, and R. Feuer.** 2005. Host and virus determinants of picornavirus pathogenesis and tropism. Nat. Rev. Microbiol. **3:**765–776.
47. **Zhu, J., M. H. Koken, F. Quignon, M. K. Chelbi-Alix, L. Degos, Z. Y. Wang, Z. Chen, and H. de The.** 1997. Arsenic-induced PML targeting onto nuclear bodies: implications for the treatment of acute promyelocytic leukemia. Proc. Natl. Acad. Sci. USA **94:**3978–3983.